Study Of Orientation Behavior of *Cissus Quadrangularis* Stem and Leaves in Albino Rat Rattus Rattus

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***Abstract*—In this study, we observed the orientation behavior of aqueous, chloroform and alcohol extract of *Cissus quadrangularis* stem and leaves in proven fertile male albino rats towards female rats (licking & anogenital Sniffing) Towards environment (climbing & exploration) towards self (nongenital grooming& genital grooming). After dosing them with 100, 250 and 500mg/kg body weight once daily for 15 days prior to the test. The result shows that Cissus *quadrangularis* stem and leaves modified orientation behavior of treated rats in that they significantly influence licking & anogenital Sniffing towards female and also increase in self-orientation as compared to control (P<0.05). However treated males possess lack of interest in external environment as indicated by reduction in exploration climbing on cage wall. Hence the further study support the folklore use of *Cissus quadrangularis* as an aphrodisiac. Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. The aqueous extract at the dose of 500 mg/ kg body weight had a pronounced effect on the orientation behavior.**

***Index Terms*—Aphrodisiac, orientation behavior, *Cissus quadrangularis*, Male albino rat, stem and leaves.**

I. INTRODUCTION

Sexual relationships are among the most significant social and biological aspects of human life. Sexual health plays a vital role in an individual's overall quality of life and well-being [1]. For the continuity of life, organisms must reproduce before death. One of the primaries aims of marriage is procreation (reproduction), as well as the sexual fulfillment of both partners. Healthy sexual functioning can enhance relationship satisfaction and boost self-esteem in humans. Aphrodisiacs have attracted human interest since ancient times due to their perceived ability to enhance sexual desire and performance.

Male impotence, commonly referred to as erectile dysfunction (ED), is a major health concern that may contribute to infertility [2]. The global incidence of ED has increased, likely due to aging populations and the rising prevalence of risk factors such as chronic illnesses (e.g., heart disease, hypertension, and diabetes mellitus), smoking, stress, alcohol consumption, drug abuse, and sedentary lifestyles. ED is defined as the consistent inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse, the inability to ejaculate, or both [3].

However, many conventional treatment options for sexual dysfunction are expensive and may not be readily accessible or affordable for a large proportion of the population [4]. For thousands of years, plants have played a crucial role in maintaining human health and enhancing quality of life. They have served as important sources of medicines, seasonings, beverages, cosmetics, and dyes [5, 6].

Historically, herbal remedies have been widely used to enhance sexual performance and increase libido. For centuries, Arab communities have relied on various herbal preparations for this purpose [7]. Similarly, in African traditional medicine—particularly in Cameroon—Zingiber officinale (ginger) and Penta diplan-drabrazzeana have been used as aphrodisiacs and for male sexual stimulation [8].

Many substances classified as aphrodisiacs are believed to cross the blood–brain barrier and stimulate regions of the central nervous system associated with sexual arousal. In addition, certain nutritional foods contribute to overall well-being, which may indirectly enhance sexual performance and libido [9].

Cissus quadrangularis is a member of the Cissus genus in the Vitaceae family. This plant is characterized as a

sprawling shrub, typically growing to a height of about 1.5 cm. Studies have shown that the alcoholic extract of this plant can aid in the rapid healing of fractures in albino rats, both when applied locally and through intramuscular methods [10]. The methanol extract (90%) and dichloromethane extract from the stem of C. quadrangularis exhibit antibacterial effects against S. aureus, E. coli, and P. aeruginosa, as well as mutagenic activity against Salmonella microsome [11-13]. Furthermore, the alcoholic extract from the aerial parts has been found to have antiprotozoal activity against Entamoeba histolytica [13]. The stem's alcoholic extract has shown effectiveness against E. coli [14]. Nevertheless, the aphrodisiac claims associated with Cissus quadrangularis by tribal communities have not been scientifically substantiated. Thus, this research was conducted to provide scientific backing for its traditional applications.

II. MATERIAL AND METHOD:

Collection of plant material

The plant *Cissus quadrangularis* was collected from Melghat region, identified and authenticated by experts from Botanical Survey of India, Pune, where a voucher specimen with herbarium accession number (SHPACIQ7) was deposited.

Animal Stock

Healthy wistar male and female albino rats of approximately 8 weeks of age and weighing 100-160 gm were purchased from Sudhakarrao Naik Institute of Pharmacy, Pusad. They were housed in a polypropylene cage, maintained at a temperature of approximately 25 ± 2 ˚C. and a photoperiod of 12 h light and 12 h dark cycle. The animals were provided with standard pelleted diet (Trimurti Lab Feeds, Nagpur) and water *ad libitum*. They were allowed a 15 days acclimatization period before the experimental session. All the experimental protocols were met with the approval of institutional Animal Ethics Committee with registration number (1060/ac/07/CPCSEA (IAEC/01/2009).

Preparation of plant extract

The stem and leaves of *Cissus quadrangularis* were collected, shade dried, cut into pieces, pulverized using an electric blender and subjected to soxhlet extraction for 24 h with distilled water (60 ˚C), chloroform (20 ˚C) and alcohol (20 ˚C). The extract was evaporated to near dryness on a water bath, weighed and stored at 4˚c in refrigerator until the experimental testing.

Preparation of test samples

Aqueous, chloroform, and alcohol extracts were suspended in 5 ml/kg of distilled water or olive oil (Figaro - refined olive oil, Spain) and given orally. A suspension of Sildenafil citrate was created by crushing a tablet of Sildenafil citrate and was administered orally at a dosage of 5 ml/kg in distilled water. (Caverta - Sildenafil citrate IP-50mg Ranbaxy, Sirmour, India).

Phytochemical analysis

The phytochemical examination of the aqueous, chloroform, and alcohol extracts from the stems and leaves of *Cissus quadrangularis* indicated the presence of alkaloids and saponins, while anthraquinone glycosides, tannins, phenolic compounds, and steroids were absent [15].

Acute toxicity study

The healthy 60 male albino rats, starved for 3- 4 h, group I was administered with the distilled water (1 ml/rat), group II-X were administered with 1000, 2500 and 5000 mg/kg dose of aqueous, chloroform and alcohol extract and subjected to acute toxicity studies. The rats were observed continuously for 2 h for behavioural, neurological and autonomic profiles and for 24 and 72 h for any lethality or death. No death was observed at highest dose (5000 mg/kg body weight) so it’s one tenth (500mg/kg) used for studies as per Organization of Economic Co-operation and Development (OECD) 423 guideline [16].

III. ORIENTATION BEHAVIOR ANALYSIS:

Male rats of proven fertility were divided into 11 groups (I-XI) consisting of 6 animals each. Rats in group I served as the control and received distil water (5 ml/kg) for 15 days. And groups II, III, IV were administrated with aqueous extract at the dose of 100,250,500 mg/kg body weight (b. w.) respectively in distilled water (5 ml/kg). Group VI-VII-administered with daily dose of chloroform extract100,250,500 mg/kg b. w. respectively in olive

oil (0.5 ml/rat, Figaro- refined olive oil, Spain). Group VIII-X- were administered with daily dose of alcohol extract100,250, 500 mg/kg b. w. respectively in olive oil (5 ml/kg), once daily at 24h intervals for 15 days. Group XI- was given 5 mg/Kg b. w. of Sildenafil citrate suspension prepared by crushing a tablet of Sildenafil citrate (Caverta -Sildenafil citrate IP-50 mg) Ranbaxy, Sirmour, India. (5 mg/kg) in distilled water, orally,1hr prior to commencement of experiment on 15^{th} day.

On the day 15 the male rats were observed for their orientation activity between 18:00 to 20:00 pm. The analysis of orientation activity was carried out and analyzed in three segments according to the method of Islam [17].

The following parameters were studied and scored for analysis of orientation activity:

Orientation towards female – (1 for every sniffing and 2 for every licking)

Orientation towards self – (1 forevery non-genital grooming and 2 for every genital grooming)

Orientation towards environment – (1 for every climbing and 2 for every exploration)

Orientation behaviour of male rats was scored using above method of scoring: The cumulative frequency of above parameter was measured.

Statistical analysis

The data are expressed as mean±SE. Statistical analysis was done by using paired and unpaired Student's t-test and one way analysis of variance (ANOVA) [18].

Result

Phytochemical analysis of the aqueous, chloroform, and alcohol extracts from the stem and leaves of *Cissus quadrangularis* revealed the presence of alkaloids, steroids, and saponins, whereas anthraquinone glycosides, tannins, and phenolic compounds were not detected. The extract of *Cissus quadrangularis* stem and leaves did not result in any toxic symptoms, such as salivation, weight loss, alterations in hair appearance, or mortality. Additionally, no changes were noted in the behavioral, neurological, and autonomic profiles at an oral dose level of 5000 mg/kg body weight in male rats. Therefore, the drug was deemed safe for further aphrodisiac evaluation. Consequently, one-tenth of this dose, specifically up to 500 mg/kg body weight, was utilized as the maximum dosage for aphrodisiac testing.

Orientation behaviour

The aqueous extracts of *Cissus quadrangularis* markedly influenced the orientation behaviour of the treated animals, which showed more attraction towards female rats. A more than two-fold enhancement in attraction towards female was noticed in the aqueous extract treated group (500mg/kg b.w.), whereas this attraction was seen to be increased two times in the chloroform and alcohol treated group (500mg/kg b.w.). However only one and half fold increase was observed in Sildenafil citrate treated group as compared to control. The behavioural assessment of rats towards self was also more pronounced in aqueous extract treated groups as compared to control group of animals. Aqueous, chloroform and alcohol extract treated males mostly showed a lack of interest in the external environment as indicated by a reduction in exploration and climbing on the cage wallas compare to control (Table 1).

Table: Effect of aqueous, chloroform and alcohol extracts of *Cissus quadrangularis* stem and leaves on orientation activities in male rats

Treatment groups	Doses (mg/kg body wt.)	Mean orientation score towards female (licking & anogenital sniffing)	Mean orientation score towards environment (climbing& exploration)	Mean orientation score towards self (nongenital grooming & genital grooming)
Control (Group I)	vehicle	6.16±0.16	6.16±0.23	7.16±0.23
Aqueous extract (Group II-IV)	100	8.83±0.24***	7.16±0.23***	9.5±0.27***
	250	11.5±0.27***	8.66±0.26***	12.5±0.26***

	500	16±0.30***	12.66±0.26***	15.16±0.23***
Chloroform extract (Group V-VII)	100	6.66±0.26*	5.16±0.23ns	8.66±0.26***
	250	8.5±0.27***	8.16±0.27***	10.66±0.26***
	500	12.33±0.26***	10.33±0.26***	14.33±0.26***
Alcohol extract (Group VIII-X)	100	6.33±0.26ns	3.33±0.26ns	7.66±0.23*
	250	7.5±0.27***	4.33±0.26*	9.33±0.26***
	500	11.66±0.26***	9.16±0.23***	12.33±0.26***
Sildenafil Citrate (Group XI)	5	10±0.16***	8.66±0.21***	10.33±0.22***

P values: * <0.1, **<.0.01, ***<0.001, when compared with control, ns= non-significant.
Values are mean±S.E. n=6.

IV. DISCUSSION

Orientation behaviour of *Cissus quadrangularis* stem and leaves:
Administration of *Cissus quadrangularis* extract (aqueous, chlololform and alcohol) at a dose of (100, 250 and 500 mg/kg b.w.) modified the orientation behavior, conclusively suggesting a better sexual performance after administration of the extracts. The results are indicative of an enhancement of the overall sexual stimulus in the body, which is considered to be very important for the treatment of sexual debility. Similar findings were reported by Subramoniam *et al.* in their study on *Trichopus zeylanicus* plant and *Lepedium meyenii* plant respectively [19]. Administration of *Anacyclus pyrethrum* extract modified the orientation behaviour in rats [20].

Eurycoma longifolia JACK modified the orientation activities of the treated male rats in that they significantly displayed more frequent and vigorous mounting, licking and anogenital sniffing towards the receptive females, and it further intensified self-orientation as reorted by the increased grooming of the genitals compared to the control. Treated males possessed a lack of interest in the external environment as indicated by a reduction in exploration, raring and climbing on the cage wall, thus supporting the folk use of *E. longifolia* JACK as an aphrodisiac.
Hence, the present study further supports the folklore use of *Cissus quadrangularis* as an aphrodisiac.

V. CONCLUSION:

In conclusion, the study validates the effectiveness of herb in improving orientation behaviour. The 500 mg/kg dose of aqueous extract shows pronounced effect on mounting frequency ensures the pure libido of the extract.

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